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Distribution of *Legionella* Species from Environmental Water Sources of Public Facilities and Genetic Diversity of *L. pneumophila* Serogroup 1 in South Korea[▽]

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A total of 560 *Legionella* species were isolated from environmental water sources from public facilities from June to September 2008 throughout South Korea. The distribution of *Legionella* isolates was investigated according to geographical region, facility type, and sample type. The genetic diversity of 104 isolates of *Legionella pneumophila* serogroup 1 (sg 1) was analyzed by sequence-based typing (SBT). *L. pneumophila* was distributed broadly throughout Korea, accounting for 85.0% of the isolates, and *L. pneumophila* sg 1 predominated in all of the public facilities except for the springs. *Legionella anisa* and *Legionella bozemanii* predominated among non-*L. pneumophila* species (48.1% and 21.0%, respectively). The second most dominant strain differed depending on the facility type: *L. anisa* was the second most dominant strain in the buildings (10.8%), *L. pneumophila* sg 5 in public baths (21.6%), *L. pneumophila* sg 6 in factories (12.0%), and *L. pneumophila* sg 7 in hospitals (13.1%). In the SBT analysis, 104 *L. pneumophila* sg 1 isolates were differentiated into 26 sequence types (STs) and categorized into 3 clonal groups (CGs) and 10 singleton STs via the eBURST V3 program. ST1, a potential founder of major CG1, was commonly distributed (48.1%). The dominant ST in hot water was ST-K1 (7, 12, 17, 3, 35, 11, 11), which was designated in this study (36.1%). The second most dominant strain differed depending on the type of facility from which the samples were obtained. The unique allelic profile of ST-K1, obtained from hot water, was not found in the European Working Group for *Legionella* Infections (EWGLI) SBT database.

Legionella species, ubiquitous Gram-negative bacteria, are found in a variety of artificial water systems, natural freshwaters, and soils. Currently, the *Legionella* genus includes 52 species and more than 70 different serogroups, and more than 20 species have been proven to be causative agents of Legionnaires' disease (LD). The species *Legionella pneumophila* accounts for approximately 90% of confirmed cases of legionellosis, and *L. pneumophila* serogroup 1 (sg 1) has been recognized as the most important agent in this regard, as that specific strain was initially implicated as the pathogen causative of LD in 1977 (15; http://www.bacterio.cict.fr/l/legionellaceae.html). The other non-*L. pneumophila* sg 1 strains, sg 2 to 15, accounted for 7.4% of cases, and *Legionella longbeachae* (3.9%) and *Legionella bozemanii* (2.4%) have also been associated with the pathogen of LD. In particular, *L. longbeachae* has been recognized as accounting for 30.4% of community-acquired *Legionella* isolates in Australia and New Zealand (53).

The most common transmission mechanism of legionellosis is the inhalation of aerosols from the water systems of artificial facilities, including large buildings, hotels, hospitals, public baths, spas, or decorative fountains contaminated by *Legionella* species (1). Therefore, hot water and water from cooling towers have been perceived as sources of infection in cases of community-acquired, nosocomially acquired, or travel-associated LD (15, 26, 31, 37, 38, 39, 41, 43). Thus, it is important from a public health perspective to continually survey environmental water systems for the presence of *Legionella* species (2, 34, 35). In particular, hot-water systems used as public baths, such as springs, spas, or tubs, have become a popular means of recreation in a lot of countries, including South Korea. The contamination of hot-water systems has gradually become recognized as an important risk factor all over the world (4, 12, 18, 23, 42, 50), as sources of legionellosis have been detected increasingly since 1982 (52) and many cases of nosocomially acquired (32, 51) and community-acquired (6, 7, 48) LD have been detected in *Legionella*-contaminated hot-water systems or hot springs.

In South Korea, several cases of nosocomial infection and community-acquired pneumonia have occasionally been reported (9, 45) since the first recognized outbreak in South Korea in 1984, which was associated with *Legionella gormanii* (27). Since 2006, the Korean National Infectious Disease Surveillance (NIDS) program (http://dis.cdc.go.kr/) has reported an average of 20 cases of LD per year (29). In South Korea, surveys of *Legionella* acquired from environmental water in public facilities such as hot springs and public baths has been gradually enhanced since 2007. An annual training program for the detection of *Legionella* species from environmental water systems and clinical specimens is currently conducted for the personnel of 16 Provincial Institute of Health and Environment locations (PIHEs) throughout South Korea. Recently, the rate of detection of environmental *Legionella* bacteria has

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TABLE 1. Distribution of samples and *Legionella* isolates from 7 geographical regions

Geographical region	Cooling tower water		Hot water		Total	
	No. of samples (%)	No. of isolates (%)	No. of samples (%)	No. of isolates (%)	No. of samples (%)	No. of isolates (%)
Seoul	316 (8.5)	4 (1.3)	187 (15.6)	51 (20.2)	503 (10.2)	55 (9.8)
Gyeonggi	784 (21.0)	21 (6.8)	157 (13.1)	12 (4.8)	941 (19.1)	33 (5.9)
Gangwon	367 (9.8)	3 (1.0)	264 (22.0)	13 (5.2)	631 (12.8)	16 (2.9)
Chungcheong	664 (17.8)	120 (39.0)	102 (8.5)	33 (13.1)	766 (15.5)	153 (27.3)
Gyeongsang	1,239 (33.2)	124 (40.3)	459 (38.2)	123 (48.8)	1,698 (34.4)	247 (44.1)
Jeolla	373 (10)	34 (11)	29 (2.4)	20 (7.9)	402 (8.1)	54 (9.6)
Jeju	93 (2.5)	2 (0.6)	4 (0.3)	0 (0.0)	97 (2.0)	2 (0.4)
Total	3,736 (100)	308 (100)	1,202 (100)	252 (100)	4,938 (100)	560 (100)

been gradually increasing (8.1% in 2006, 9.4% in 2007, and 10.3% in 2008).

The principal objectives of this study were to assess the current distribution of *Legionella* species from environmental water sources from public facilities such as buildings, hotels, public baths, springs, hospitals, or factories throughout South Korea. Additionally, the molecular typing of *L. pneumophila* sg 1 isolates was conducted using sequence-based typing (SBT) to assess the genetic diversity among the isolates.

MATERIALS AND METHODS

Sampling and bacterial isolates. Sixteen PIHEs (in Seoul, Incheon, Gyeonggi, Gangwon, Daejeon, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gwangju, Gyeongbuk, Gyeongnam, Daegu, Ulsan, Busan, and Jeju) participated in a survey of *Legionella* in public facilities throughout South Korea from June to September 2008.

A total of 4,938 environmental water samples were collected largely from cooling towers and hot-water systems in large buildings, hospitals, or public baths in each region. The sampling was conducted via identical sampling protocols in all geographic regions. One liter of water samples from cooling towers, faucets, or showers was collected in sterile specimen bottles. The numbers of samples and isolates depended on the number of facilities located in each region. A total of 560 *Legionella* isolates from 13 out of 16 PIHEs were then sent to our laboratory in order to confirm the serological identification.

Identification of *Legionella* species. To reconfirm the identification of the *Legionella* species, all of the collected strains were subcultured onto BCYE agar without L-cysteine or BCYE agar with L-cysteine. After the colonies growing only on BCYE agar were selected, *Legionella* species were confirmed via PCR using primers for *Legionella* genus-specific 16S rRNA and *L. pneumophila* species-specific *mip* genes (10, 24). Serological identification was then performed using a latex agglutination test (Oxoid, England), an antiserum kit (Denka, Japan), or a direct fluorescent-antibody assay (DFA) kit (m-Tech) for *Legionella* spp. Isolates not identified by serological methods were confirmed via 16S rRNA, *mip*, or *rpoB* gene amplification and comparative sequence analyses using the NCBI database (24, 25, 28).

SBT and allelic diversity analyses. Genotyping was conducted via the standard sequence-based typing (SBT) method of the European Working Group for *Legionella* Infections (EWGLI) using 7 genes (*flaA*, *pilE*, *asd*, *mip*, *mompS*, *proA*, and *neuA*) (16, 40). Nucleotide analyses used the SBT database available on the EWGLI website (http://www.ewgli.org/), and the sequences were compared with those in the SBT database, which are also available on the website (http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php). Additionally, sequence types (STs) that were not available in the EWGLI SBT database were represented alphanumerically in this study (e.g., ST-K1).

The clonal complexes were analyzed using eBURST V3 (http://eburst.mlst.net), and clusters of related STs that descended from a common ancestor were defined as clonal groups (CGs). Single genotypes corresponding to no CG were defined as singletons.

Statistical analyses. All calculations were conducted using SPSS 12.0 software (SPSS, Inc., Chicago, IL). The chi-square test was used to compare the proportional distributions of *Legionella* species according to environmental water source type.

RESULTS

Collected samples and isolates. A total of 560 isolates were classified into 7 regions based on geographic location (Table 1), and the number of isolates was proportional to the number of collected samples. In particular, samples collected from the Gyeongsang region accounted for 34.4% of the overall samples, and the isolates from this region accounted for 44.1% of the total. For this reason, more institutions (Gyeongbuk, Gyeongnam, Daegu, Ulsan, and Busan) in this region than in other regions participated in this study. Although the isolates analyzed in this study were not selected in one region, the possibility that a bias was induced by regional differences in the number of strains could not be dismissed.

During sample collection, water characteristics such as temperature, pH, and residual chlorine concentration were usually not measured in this study.

Serological distribution of *Legionella* species. A total of 560 isolates of *Legionella* were isolated from buildings (36.3%), public baths (28.9%), hospitals (25.9%), factories (4.5%), springs (2.5%), and hotels (2.0%). Fifty-five percent of the total samples were collected from cooling tower water, and the rest of the samples were collected from hot water. Among the 560 isolates, the 479 *L. pneumophila* isolates statistically predominated (85.5%), whereas *Legionella* species other than *L. pneumophila* accounted for 14.5% of the total. Among the 479 *L. pneumophila* species, the sg 1 strain accounted for 262 (54.7%), whereas strains sg 5, sg 6, sg 7, sg 3, and sg 10 accounted for 11.9%, 11.5%, 6.3%, 4.8%, and 2.7%, respectively (Fig. 1A). Thirty-nine *Legionella anisa* isolates were isolated, making *L. anisa* the dominant species (48.1%) among 81 non-*L. pneumophila* species, followed by *L. bozemanii*, which accounted for 21.0%. A total of 23.5% of the total isolates remained unidentified, despite genetic analyses using the 16S rRNA, *mip*, or *rpoB* genes (Fig. 1B).

Analysis of geographic distribution of *Legionella*. *L. pneumophila* sg 1 was prevalent in the 7 regions throughout South Korea (Fig. 2), and *L. pneumophila* sg 5 was represented in Seoul (14.5%), Gyeonggi (18.2%), Chungcheong (7.8%), Gyeongsang (9.3%), and Jeolla (14.8%) but not Gangwon or Jeju. *L. pneumophila* sg 6 was detected in Seoul (9.1%), Gyeonggi (12.1%), Gangwon (6.3%), Chungcheong (7.8%), Gyeongsang (11.3%), and Jeolla (9.3%) but not Jeju. *L. anisa* was isolated from 4 regions, all except Seoul, Gyeonggi, and Jeju,

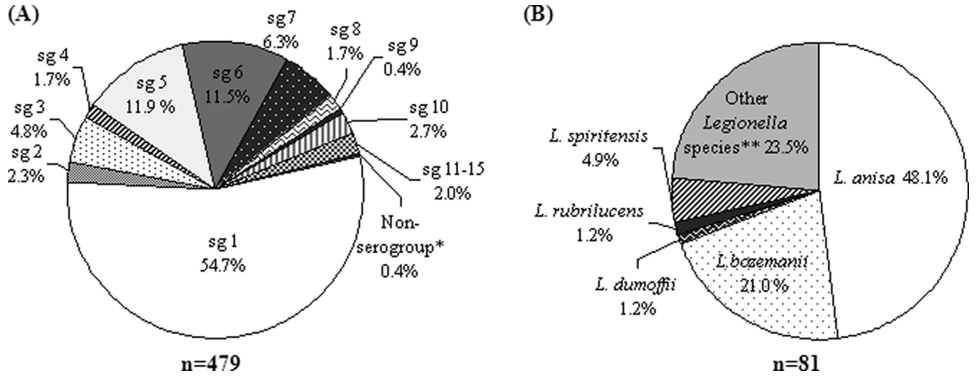

FIG. 1. Distribution of *Legionella* species from environmental water sources in South Korea for 2008. (A) *L. pneumophila*; (B) Non-*L. pneumophila* species. "Non-serogroup*" comprised *L. pneumophila* isolates not identified as members of any serogroup, and "other *Legionella* species**" comprised *Legionella* isolates not identified as members of any known species. sg 11, sg 13, sg 14, and sg 15 accounted for 0.2% each, and sg 12 accounted for 0.8%.

and *L. bozemanii* was also not detected in the Seoul, Gyeonggi, and Jeju regions.

***Legionella* species according to facility type.** *L. pneumophila* sg 1 accounted for 46.8% (262/560) of the total isolates and predominated in facilities such as buildings, public baths, hospitals, factories, and hotels although not in springs (Fig. 3). The distribution rates of *L. pneumophila* strains other than sg 1 depended on the facility types; *L. pneumophila* sg 5 prevailed in public baths (21.6%), *L. pneumophila* sg 6 in springs and factories (21.4% and 12.0%, respectively), and *L. pneumophila* sg 7 in hospitals (13.1%). However, it was unreasonable to conclude that *L. pneumophila* sg 6 predominated in springs, as only 14 isolates were collected from springs. Among other non-*L. pneumophila* species, *L. anisa* accounted for 10.8% in buildings.

***Legionella* species in cooling tower water and hot water.** In order to determine whether the distribution of *Legionella* species depended on the sample type, the species and serogroup

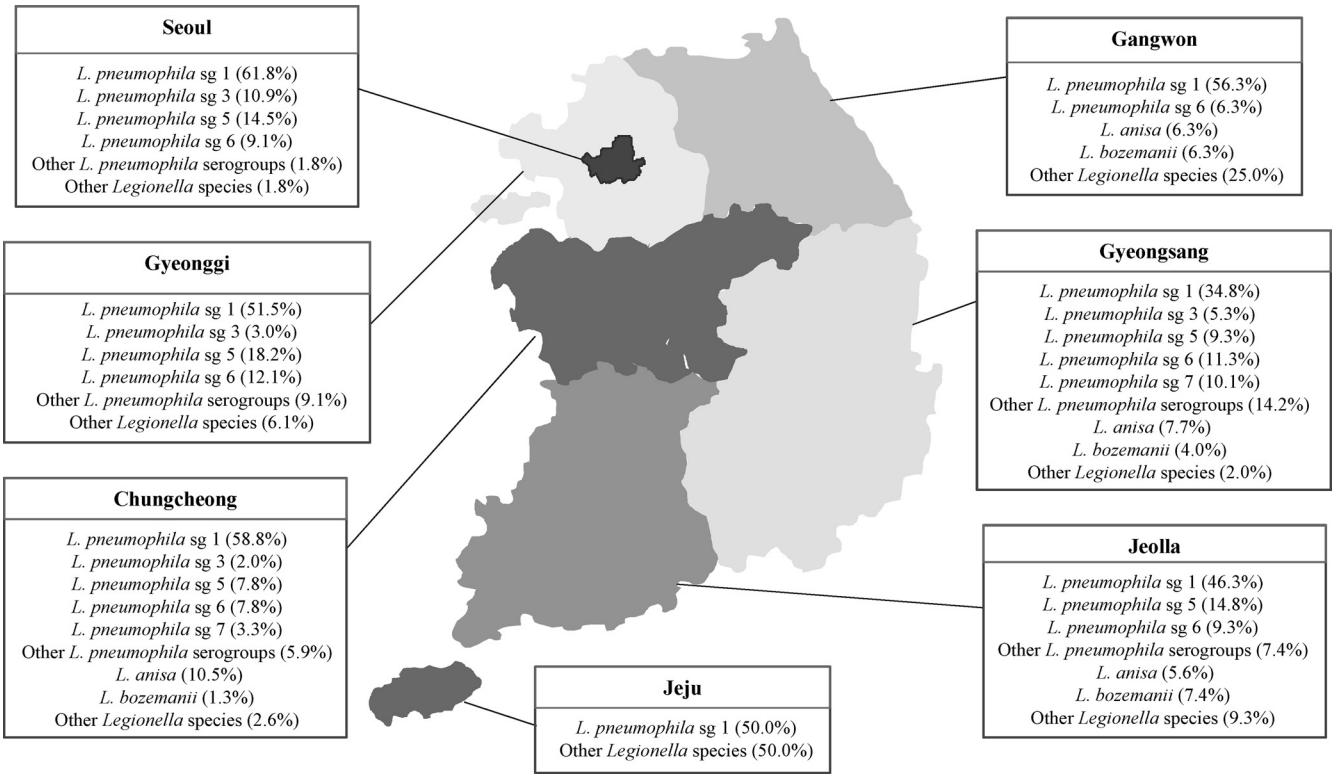

FIG. 2. Distribution of *Legionella* species isolated from environmental water sources from 7 geographic regions in South Korea: Seoul ($n = 55$), Gyeonggi ($n = 33$), Gangwon ($n = 16$), Chungcheong ($n = 153$), Gyeongsang ($n = 247$), Jeolla ($n = 54$), and Jeju ($n = 2$). "Other *L. pneumophila* serogroups" comprised sg 7 to sg 15, and "other *Legionella* species" comprised *L. dumoffii*, *L. rubrilucens*, *L. spiritensis*, and *Legionella* isolates not identified as members of any known species.

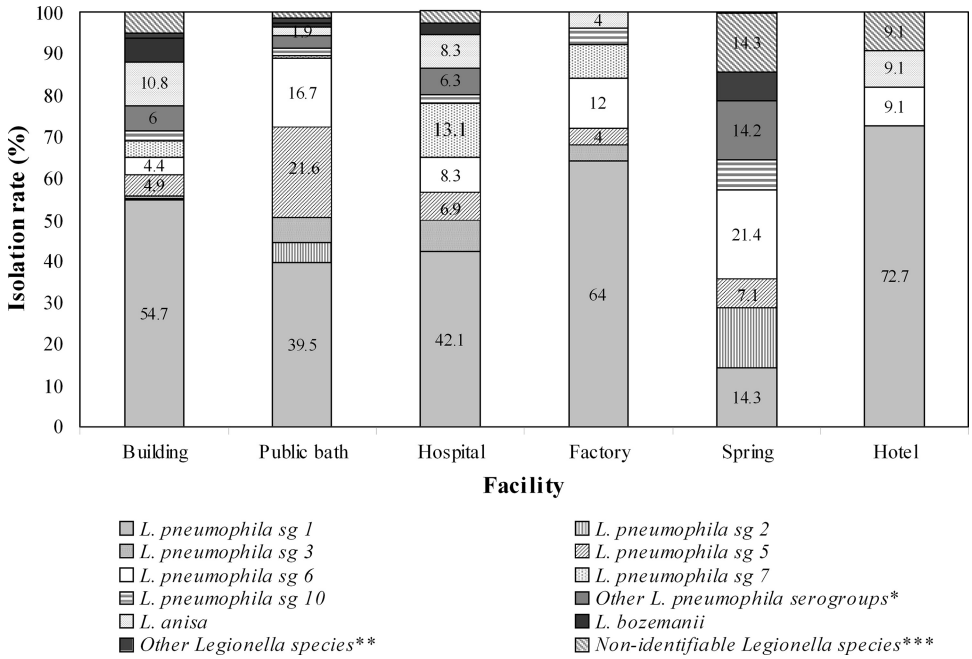

FIG. 3. Distribution of *Legionella* species from environmental water sources according to facility type ($n = 560$). "Other *L. pneumophila* serogroups*" comprised sg 4 (2.0% in buildings and 2.8% in hospitals), sg 8 (2.5% in buildings, 1.2% in public baths, and 0.7% in hospitals), sg 9 (0.6% in public baths and 7.1% in springs), sg 11 (0.6% in public baths), sg 12 (0.5% in buildings, 0.6% in public baths, 0.7% in hospitals, and 7.1% in springs), sg 13 and sg 14 (0.5% each in buildings), sg 15 (0.7% in hospitals), and *L. pneumophila* isolates not identified as members of any serogroup (1.4% in hospitals). "Other *Legionella* species**" comprised *L. dumoffii* and *L. rubrilucens* (0.6% each in public baths) and *L. spiritensis* (1.5% in buildings and 7.1% in springs). "Non-identifiable *Legionella* species***" comprised *Legionella* isolates not identified as members of any known species.

distributions were compared between the 276 isolates from the cooling tower water and the 252 isolates from hot water. The predominant strain in the cooling tower water and the hot water was *L. pneumophila* sg 1 (54.3% and 37.3%, respectively); the secondarily dominant strains depended on the sample type. *L. anisa* accounted for 9.4% of isolates collected from cooling towers, and *L. pneumophila* sg 5 accounted for 17.1% of the hot-water samples. In our comparative analysis of distribution between the cooling tower water and the hot water acquired from hospitals, *L. pneumophila* sg 1 was the predominant strain in both sample types, and the second most dominant strains depended on the sample type: *L. pneumophila* sg 7 was the second most dominant strain in cooling towers (20.8%), and *L. pneumophila* sg 3 in hot water (13.2%). *L. anisa* was the strain of quaternary dominance (5.2%) in the cooling tower samples and of tertiary dominance (11.8%) in the hot-water samples (Fig. 4).

Genetic diversity. For SBT, among 262 isolates of *L. pneumophila* sg 1, 104 isolates were selected randomly, and these isolates were differentiated by SBT into 26 different sequence types (STs). ST1 (1, 4, 3, 1, 1, 1, 1), as the predominant type, accounted for 48.1% and was distributed commonly throughout the majority of facilities and regions (Table 2). ST36 (3, 4, 1, 1, 14, 9, 1) of *L. pneumophila* sg 1 (Philadelphia-1; ATCC 33152) was found only in one of the hot-water samples, and the profile of ST-K1 to -K14 could not be found in the EWGLI SBT database. According to the results of our eBURST analysis, 26 STs belonged to 3 CGs and 10 singleton STs (Table 2). In 3 CGs, CG1, the prevalent clonal group, included ST1, ST-K2, ST296, ST304, ST-K5, ST-K3, ST-K4, and ST-K8; the putative ancestor of CG1 was predicted to be ST1. The reminders were CG2 (ST50, ST159, ST-154, and ST-K14) and CG3 (ST59, ST363, ST-K1, and ST-K11). Among the 10 singleton STs, ST45 was detected in 3 isolates.

In our comparative analysis of the SBT distribution of the isolates according to sample type (Fig. 5), ST1 was the predominant type in isolates from the cooling tower water (67.6%, 46/68) and ST-K1 (7, 12, 17, 3, 35, 11) was the dominant type in the hot-water samples (36.1%). However, ST-K3, -K4, -K5, -K6, -K7, and -K14 were found only in isolates from the cooling tower water, and ST-K2, -K8, -K9, -K10, -K11, -K12, and -K13 were found only in the hot-water isolates.

DISCUSSION

The results of this study showed that the ecology of *Legionella* species differed between the water of cooling towers and the hot-water samples collected from public facilities. With regard to sample type, *L. pneumophila* sg 1 was identified as a major strain (54.3% in water of cooling towers and 37.3% in the hot-water samples). Among facilities such as buildings, hospitals, public baths, and factories, 66.5% of isolates from cooling tower water were *L. pneumophila* sg 1, compared to only 41% of the isolates collected from hot water ($P < 0.001$) (Table 3). Among the hospitals, 94.8% of the isolates collected from cooling tower water were identified as *L. pneumophila*, compared to only 76.5% of the isolates collected from hot water ($P = 0.002$) (Table 4). Our results differed from those

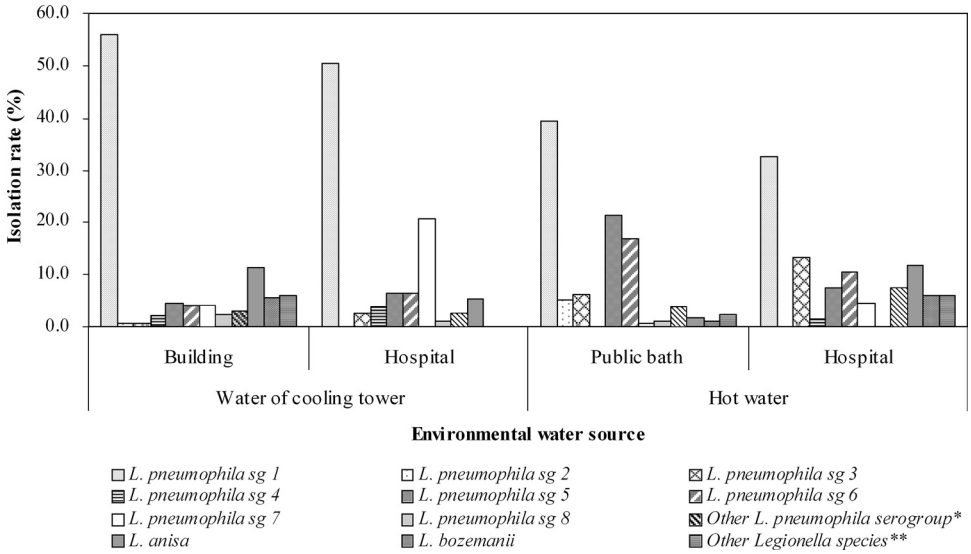

FIG. 4. Comparison of rates of isolation of *Legionella* species from cooling tower water ($n = 276$) and hot water ($n = 228$) in facilities such as buildings, hospitals, and public baths. "Other *L. pneumophila* serogroups*" comprised sg 9 to 15 and *L. pneumophila* isolates not identified as members of any serogroup. "Other *Legionella* species**" comprised *L. dumoffii*, *L. rubrilucens*, *L. spiritensis*, and *Legionella* isolates not identified as members of any known species.

reported in earlier studies of public facilities. Among the cooling systems and hot-water systems of Spanish health care facilities (42), the predominant species, *L. pneumophila*, accounted for 85.1% and 91.7% of the total isolates, respectively. With regard specifically to *L. pneumophila* strains, sg 1 was the most frequently detected strain, at 88.3% and 81.8% in cooling towers and hot-water tanks, respectively. Another study of cooling towers conducted in China (31) reported that *L. pneu-*

TABLE 2. Distribution of clonal groups from 26 SBT profiles for *L. pneumophila* sg 1 isolates ($n = 104$) in South Korea

Clonal group	ST	Alleic profile							No. of isolates (%)
		flaA	*pilE*	*asd*	*mip*	*mompS*	*proA*	*neuA*	
CG1 ($n = 61$)	1	1	4	3	1	1	1	1	50 (48.1)
	K2	1	4	3	1	1	1	4	2 (1.9)
	296	1	4	3	1	1	1	11	2 (1.9)
	304	1	4	1	1	1	1	1	3 (2.9)
	K5	1	12	3	1	1	1	1	1 (1.0)
	K3	1	4	3	1	1	3	1	1 (1.0)
	K4	1	10	1	1	1	9	1	1 (1.0)
	K8	3	10	1	1	1	9	3	1 (1.0)
CG2 ($n = 21$)	59	7	6	17	3	13	11	11	4 (3.8)
	363	7	6	3	3	13	11	11	2 (1.9)
	K1	7	12	17	3	35	11	11	14 (13.5)
	K11	7	10	17	3	13	14	11	1 (1.0)
CG3 ($n = 10$)	150	11	14	16	1	15	13	1	4 (3.8)
	159	11	14	16	1	15	13	2	2 (1.9)
	154	11	14	16	16	15	13	2	3 (2.9)
	K14	11	14	3	1	15	13	1	1 (1.0)
Singletons ($n = 12$)	K7	2	23	17	3	9	4	6	1 (1.0)
	K6	2	6	14	10	1	4	11	1 (1.0)
	45	5	1	22	26	6	10	12	3 (2.9)
	K13	10	10	7	28	8	18	6	1 (1.0)
	42	4	7	11	3	11	12	9	1 (1.0)
	K12	10	6	7	21	16	18	9	1 (1.0)
	36[a]	3	4	1	1	14	9	1	1 (1.0)
	22	2	3	6	10	2	1	6	1 (1.0)
	K10	6	10	15	14	21	7	11	1 (1.0)
	K9	6	10	1	3	19	4	11	1 (1.0)

[a] ST36 represents the SBT profile of *L. pneumophila* sg 1 (Philadelphia-1; ATCC 33152).

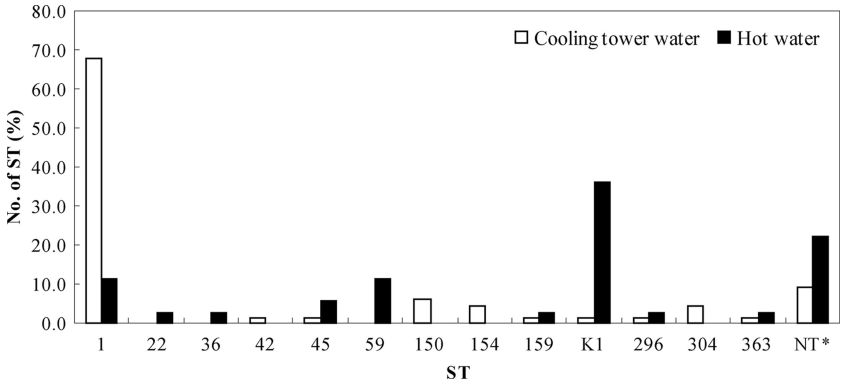

FIG. 5. Comparison of SBT profiles of *L. pneumophila* sg 1 isolates from cooling tower water ($n = 68$) and hot water ($n = 36$). The nontypeable (NT*) STs, which could not be found among the EWBLI SBT data, were ST-K2, ST-K3, ST-K4, ST-K5, ST-K6, ST-K7, and ST-K14 for cooling tower water (1.5% each) and ST-K8, ST-K9, ST-K10, ST-K11, ST-K12, ST-K13, and ST-K14 for hot water (2.8% each).

mophila sg 1 accounted for 82% (155/189) of the isolates, followed by *Legionella micdadei* (23.3% [44/189]). However, in a British analysis of unrelated environmental isolates, *L pneumophila* sg 1 accounted for 42.7% of the total isolates, followed by 12% and 7.7% for sg 6 and sg 5, respectively (19). In another study, designed to determine the clinical and environmental distribution of *Legionella* in France, the frequency of *L. pneumophila* sg 1 isolates from environmental water (75.6%) was lower than that observed for the clinical samples (98.8%), thus suggesting that environmental predominance was unrelated to more-efficient intracellular growth or higher infectivity (11). Some authors have made the point that non-*L. pneumophila* species with higher prevalence in environmental water samples than in the clinical samples may prove to be less pathogenic than *L. pneumophila*. This hypothesis has been recognized for the majority of confirmed infections by non-*L. pneumophila* species occurring in immunosuppressed patients (36, 46). The other studies have identified *L. anisa* as the cause of Pontiac fever (13, 14) or hospital-acquired LD (5, 14, 43). The detection of *L. anisa* in water samples should be considered an indication that the water system has been colonized by *Legionella* species, including *L. pneumophila* (49, 53). The other *Legionella* species may prove important in the etiology of community-acquired pneumonia, thus underscoring the need for diagnostic studies, including culture, serology, urinary antigen testing, or gene detection for *Legionella* species other than *L. pneumophila* sg 1 (33, 53).

As discussed in a previous publication (20), SBT is a more powerful tool than pulsed-field gel electrophoresis (PFGE) for the subtyping of *L. pneumophila* strains. The use of SBT data from different countries, then, constitutes a technically uncomplicated and relatively easy method for strain subtyping, especially compared to other contemporary techniques.

ST1 (1, 4, 3, 1, 1, 1) is distributed broadly throughout the world (3, 8, 20, 44) and was the predominant profile in this study. However, the profile of ST-K1 (7, 12, 17, 3, 35, 11) was not detected in the HPA/EWGLI SBT database or in any other studies. ST150 (11, 14, 16, 1, 15, 13) has previously been reported to occur in France (17). Additionally, 13 STs (ST-K2 to -K14) identified as unique types in this study had new allelic profiles.

The 104 isolates of *L. pneumophila* sg 1 used in SBT were not identified as members of subgroups by use of monoclonal antibodies (MAbs) from the Dresden Panel (21, 22). So, comparison of relationships between subgroups by MAbs and STs, as was the case in other studies, could not be demonstrated in this study.

The 194 clinical isolates of *L. pneumophila* sg 1 isolated from Ontario, Canada, from 1978 to 2007 comprised 62 STs, and the population of STs was highly diverse. ST36, ST42, ST45, and ST59 were identified as members of cluster II and were also found in the singletons of this study (except for ST59); however, ST150, ST154, ST159, ST296, ST304, and ST363 in this study were not found among the 62 STs (47).

In conclusion, our results demonstrated that the proportional populations of environmental isolates of *Legionella* species isolated from public facilities differed according to the types of facility or sample assessed as well as the geographical locations of the facilities. Additionally, our findings revealed several unique allelic profiles of STs and showed that ST1 of *L. pneumophila* sg 1 was the prevalent sequence type in South Korea. Routine monitoring of environmental water for *Legio-*

TABLE 3. Comparative distribution of *Legionella* species between water in cooling towers and hot water in facilities such as buildings, hospitals, public baths, and factories ($n = 560$)

Sample source	No. (%) of *Legionella* isolates			No. (%) of *L. pneumophila* isolates		
	Total	*L. pneumophila*	Non-*L. pneumophila*	Total	sg 1	Non-sg 1
Cooling tower water	308	257 (83.4)	51 (16.6)	257	171 (66.5)	86 (33.5)
Hot water	252	222 (88.1)	30 (11.9)	222	91 (41.0)	131 (59.0)
Total	560	479 (85.5)	81 (14.5)	479	262 (54.7)	64 (51.2)

TABLE 4. Comparative distribution of *Legionella* species between water in cooling towers and hot water in hospitals (n = 145)

Sample source	No. (%) of *Legionella* isolates			No. (%) of *L. pneumophila* isolates		
	Total	*L. pneumophila*	Non-*L. pneumophila*	Total	sg 1	Non-sg 1
Cooling tower water	125	73 (94.8)	4 (5.2)	73	39 (53.4)	34 (46.6)
Hot water	20	52 (76.5)	16 (23.5)	52	22 (42.3)	30 (57.7)
Total	145	125 (86.2)	20 (13.8)	125	61 (64)	64 (51.2)

nella species is expected to prove helpful in efforts to reduce the bacterial contamination of water systems and is also expected to facilitate the development of a more active prevention strategy for LD. Additionally, further study will require that the focus be kept on correlation analysis by clustering between environmental and clinical isolates of *Legionella* species. Thus, the findings of this study highlight the importance of understanding the epidemiology and ecology of *L. pneumophila* from public facilities in terms of public health; in this regard, our findings corroborate and reinforce the recommendations made in several previous studies (2, 15, 35).

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